REPORT DOCUMENTATION PAGE

Form Approved
OMB No. 0704-0188

1. AGENCY USE ONLY *(Leave blank)*	2. REPORT DATE	3. REPORT TYPE AND DATES COVERED
	1 Jan 1999	1 July 1995 - 31 Dec 1998

4. TITLE AND SUBTITLE
The biochemistry of primary attachment in the serpulid larvae Hydroides elegans
(ONR serpulid)

5. FUNDING NUMBERS
G: N00014-95-1-1015
PR: 99PR02862-00

6. AUTHOR(S)
J. Herbert Waite

7. PERFORMING ORGANIZATION NAME(S) AND ADDRESS(ES)
University of Delaware
College of Marine Studies

8. PERFORMING ORGANIZATION REPORT NUMBER

9. SPONSORING / MONITORING AGENCY NAME(S) AND ADDRESS(ES)
Office of Naval Research, Code 1141 SB
800 N. Quincy Street
Arlington, VA 22217

10. SPONSORING / MONITORING AGENCY REPORT NUMBER

11. SUPPLEMENTARY NOTES
—

12a. DISTRIBUTION / AVAILABILITY STATEMENT
Distribution unlimited

12b. DISTRIBUTION CODE

13. ABSTRACT *(Maximum 200 words)*

The serpulid polychaete, Hydroides elegans, is a prominent fouling organism in tropical marine waters. Fouling is first caused by the formation of an unmineralized primary tube and thread by settling larvae. This is replaced by a mineralized secondary attachment tube during metamorphosis. Biochemical analysis of the primary and secondary tubes suggests a composition rich in acidic amino acids and glycine. Dopa is transiently present in new growth. The latter finding, particularly, may indicate a functional similarity to the adhesive proteins of mussels, ascidians, and sabellariids.

14. SUBJECT TERMS
Fouling, Hydroides, Attachment proteins

15. NUMBER OF PAGES
4

16. PRICE CODE

17. SECURITY CLASSIFICATION OF REPORT	18. SECURITY CLASSIFICATION OF THIS PAGE	19. SECURITY CLASSIFICATION OF ABSTRACT	20. LIMITATION OF ABSTRACT
Unclassified	Unclassified	Unclassified	SAR

FINAL REPORT

GRANT #: N00014-95-1-1015

PRINCIPAL INVESTIGATOR: Herbert Waite

INSTITUTION: University of Delaware; Subcontractor: University of Hawaii (submitting a separate report).

GRANT TITLE: The biochemistry of primary attachment in the serpulid larvae *Hydroides elegans*.

AWARD PERIOD: 1 July 1995-31 Dec 1998

OBJECTIVE: To identify the proteins of primary attachment in the fouling polychaete *Hydroides elegans*.

APPROACH: There were several approaches: 1. Originally our approach was to extract and purify proteins from the primary tube of H. elegans (provided by Dr. M. Hadfield at Hawaii);. 2. Later efforts shifted to isolation of adhesion-related redox-active molecules from presettlement-competent larvae. 3. Finally, we tried to characterize proteins/peptides from juvenile tubes with the hope of tracing their expression back to primary attachment. The general approach was to release peptides from the insoluble attachment structure by proteolytic treatment; to purify and sequence the liberated peptides, and then, with degenerate oligonucleotide primers based on peptide sequences, to use RT-PCR strategies to derive first partial and then complete cDNA sequences. The protein/peptide chemistry was done at Delaware: the molecular work was to be done at Hawaii.

ACCOMPLISHMENTS: Using a micromanipulator, we manually dissected out enough primary tubes (~20) for an amino acid analysis which suggests a aspartic acid/glutamic acid/glycine rich protein. The same three amino acids plus serine dominated the composition of primary threads. Our efforts to characterize individual proteins or peptides from the primary tubes were limited for two reasons: inadequate tube material (<50µg) and inability to separate larvae from the primary tubes.Since the primary tubes and associated threads were redox-active with nitroblue tetrazolium (NBT - an indicator for quinones), we looked for expression of redox-active molecules in larvae at 2, 3 and 4 days of development post fertilization. Attachment competency usually begins at 3-4 days. Expression of redox-active components was highest at 2-3 days. Proteins were extracted from 2-day old larvae using 5% acetic acid, separated by C-18 HPLC and assayed by reaction with NBT. A peak eluting at 30 min reacted strongly with NBT. Sequence and mass were not obtainable due to heterogeneity and inadequate material. The final strategy to isolate proteins/peptides from demineralized juvenile/adult tubes did offer slightly more working material (100 mg), however, the yield of soluble peptides was always less than 0.1% of the starting material. We tried several proteolytic treatments: pepsin, chymotrypsin, lys C-endoproteinase, trypsin and cyanogen bromide which consistently liberated 24, 5, 6, 8 and 1 peak(s), respectively, by C-18 HPLC. Most

were Glu, Gly and Asp rich. Only two gave clean Edman sequence: the CNBr treatment produced Tyr. This suggests that many of the proteins had a C-terminal sequence Met-Tyr. Lys-C endopeptidase produced many peaks by HPLC but one was a clean peptide: DAEDDDD. Trypsinization of dissected new growth increments from adult tubes at Hawaii in Feb 1997 gave a strong indication of DOPA: Highest levels in C-18 HPLC peaks approached 1-2 mol %.

CONCLUSIONS: The adhesive proteins present in the primary and juvenile tubes of post-larval Hydroides elegans are probably different gene products but have significant similarities: Both contain quinone-like redox activity and both have amino acid compositions rich in aspartic/glutamic acid and glycine. Peptides released by proteolysis from the juvenile and adult tubes contain 3,4-dihydroxyphenylalanine (DOPA). Adhesive protein constituents of juvenile tubes are completely insoluble. Future efforts would do well to concentrate on isolating and characterizing soluble DOPA-containing precursor proteins from adult worms, then use immunochemical and sequence resources to determine life history-specific expression and distribution patterns. We recommend in the strongest possible terms that future investigations of Hydroides attachment biochemistry be conducted exclusively at the sites of worm collection. This is based on our experience with the rapidity of cement maturation and the chemical instability of proteins associated with attachment.

SIGNIFICANCE: Like mussels, sabellariids, tunicates and gorgonian corals, adhesion in serpulids may be based on DOPA-proteins. Although a significant base of sequence information has yet to be compiled, proteins from the tubes of Hydroides appear to be rather acidic.

PATENT INFORMATION: None.

AWARD INFORMATION: Waite was awarded the Maxwell & Mildred Harrington Professorship in July, 1998.

PUBLICATION AND ABSTRACTS:

Warren, K., Coyne, K.J., Waite, J.H., and Cary, S. C. (1998). Use of methacrylate de-embedding protocols for *in situ* hybridizations on semithin plastic sections with multiple detection strategies. *J. Histochem. Cytochem.* **46**, 149-156.

Vreeland, V., Waite, J.H., and Epstein, L. (1998). Polyphenols and oxidases in substratum adhesion in marine algae and mussels. *J. Phycol.* **34**, 1-8.

Taylor, S.W. & Waite, J.H. (1997). Marine adhesives. In *Protein-Based Materials*, K. McGrath & D. Kaplan, eds, Birkhäuser, Basel & Boston, p. 217-250.

Taylor, S.W. & Waite, J.H. (1998). Occurrence of dihydroxyproline in proteins and peptides. In *Prolyl Hydroxylase, Protein Disulfide*

Isomerase, and Other Structurally Related Proteins (N.A. Guzman, ed.) Marcel Dekker, NY p. 97-108.